



Banks, P., Burroughs, A., Barker, G., Brown, J. T., Warburton, C., & Bashir, Z. (2015). Disruption of hippocampal–prefrontal cortex activity by dopamine D2R-dependent LTD of NMDAR transmission: NMDARs control hippocampal-PFC synaptic transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 112(35), 11096–11101. <https://doi.org/10.1073/pnas.1512064112>

Peer reviewed version

Link to published version (if available):  
[10.1073/pnas.1512064112](https://doi.org/10.1073/pnas.1512064112)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

## University of Bristol - Explore Bristol Research

### General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:  
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

# Supporting Information

## SI Materials and Methods

**General Surgical procedure.** All surgical procedures were performed on male Lister-hooded rats which weighed 300-400 g at the start of the procedure. Each rat was anaesthetised with isoflurane (induction 4%, maintenance 2–3%) and secured in a stereotaxic frame with the incisor bar set at 3.3 mm below the interaural line. Injections of tracer or neurotoxin were made through burr holes at the co-ordinates outlined below. At the end of surgery each animal received fluid replacement therapy (5ml of saline containing 1 % glucose s.c.) and analgesia (0.05 ml Vetgesic i.m.).

**Hippocampal tracer injection.** Anterograde tracer (200 nl dextran-conjugated AlexaFluor488 4% in phosphate-buffered saline (PBS), Life Technologies, Paisley, UK) was injected into the ventral hippocampus (-6.5mm from bregma, -4.5mm from midline, 6.4mm below dura at an angle of 10° to the vertical plane) by pressure injection using an UltraMicroPump 3 (World Precision Instruments) fitted with a 5 µl Hamilton syringe with a 33 gauge needle at a rate of 50 nl/min, with the needle left in situ for 10 min post-injection. Animals were anaesthetised with sodium pentobarbital 7 days later and transcardially perfused with PBS followed by 4% paraformaldehyde and postfixed in paraformaldehyde for 24 h then transferred to 30 % sucrose in 0.2 M phosphate buffer for 48 h before making coronal sections (40 µm) on a cryostat. Sections were mounted with Vectashield mounting medium (Vector Labs) containing 1.5 µg/ml of DAPI. Images were obtained using a Leica DFC3000 FX camera mounted on a Leica DM5000B microscope with Leica GFP and A4 filter sets used to observe AlexaFluor488 and DAPI, respectively.

**Hippocampal lesions.** Unilateral hippocampal excitotoxic lesions were made in six 300-400g rats by making a series of injections of NMDA as indicated in Table 1. Modified coronal slices were cut, as detailed below, 21-28 days after the lesions and electrophysiological recordings were made from both hemispheres of PFC. The remaining tissue was fixed in paraformaldehyde for 48 h, transferred to 30 % sucrose in 0.2 M PBS for 48 h and sectioned at 40 µm on a cryostat before staining with cresyl violet. To determine the extent of the lesions the remaining hippocampal tissue was measured in each hemisphere (Leica Qwin 3) in every fifth section between – 1.9 and -6.3 mm relative to bregma, and the remaining hippocampal

tissue from the lesioned hemisphere expressed as a percentage of hippocampal tissue in the opposite hemisphere of the same sections.

### **Slice preparation**

Animals were anaesthetised with isoflurane and decapitated. The brain was rapidly removed and placed in ice-cold (2-4°C) oxygenated (95 % O<sub>2</sub>-5 % CO<sub>2</sub>) artificial cerebrospinal fluid (aCSF) containing (mM): 124 NaCl, 3 KCl, 26 NaHCO<sub>3</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 1 MgSO<sub>4</sub>, 10 D-glucose and 2 CaCl<sub>2</sub>. The brain was cut at an 11° modified coronal angle as described previously for the mouse (Parent et al 2009) using a custom brain matrix (Zivic Instruments, Pittsburgh, USA) and slices cut at 400 µm using a vibratome before storing in room temperature aCSF for ≥ 1h before use.

### **Electrophysiology**

Slices equivalent to +2.7 to +2.2 mm from bregma (Paxinos & Watson 1998) were transferred to a submerged recording chamber and perfused with aCSF at a rate of 2 ml/min. For voltage-clamp experiments layer 5 neurons were patch clamped in the whole-cell configuration using borosilicate glass (GC150F-10, Harvard Apparatus, UK) electrodes 2-6 MΩ filled with a cesium methylsulfonate based solution (in mM: 130 CsMeSO<sub>4</sub>, 8 NaCl, 10 HEPES, 0.5 EGTA, 4Mg-ATP, 0.3 Na-GTP, 5 QX-314-Cl, pH 7.25, 280-295 mOsm) at room temperature. Recordings were obtained using an Axon Multiclamp 700B amplifier (Molecular Devices) and WinLTP v1.11b (Anderson and Collingridge, 2007) or pClamp 10 (Molecular Devices) acquisition software, filtered at 4 kHz and digitised at 20 kHz (Digidata 1322A, Molecular Devices) without liquid junction potential correction. Cells where series resistance exceeded 30 MΩ or changed > 20 % were discarded. A concentric bipolar stimulating electrode (CBAPB50, FHC) was placed on the hippocampal fibre bundle and synaptic responses evoked using 0.1 ms constant-current pulses. Picrotoxin (50 µM) and NBQX (5 µM) were bath applied and cells were held at -40 mV to isolate EPSC<sub>NMDA</sub>. Activity dependent LTD was induced by delivery of 300 pulses at 5 Hz at baseline stimulus intensity.

Current clamp recordings were performed at 31 ± 1°C using a potassium gluconate based intracellular solution (in mM: 145 K-gluconate, 5 NaCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, 0.3 Na-GTP, pH 7.25, 280-295 mOsm) and were acquired using

pClamp10.2 software (Molecular Devices) low-pass filtered at 4 kHz and digitised at 100 kHz. Cells were held at -70 mV by injecting constant current (liquid junction potentials corrected post-hoc) and single hippocampal-PFC EPSPs were evoked at 0.1 Hz before delivering synaptic trains of 10 stimuli delivered at 20, 50 and 100 Hz. In some experiments NMDA receptors were blocked pharmacologically by bath applying 50  $\mu$ M D-AP5 for 10 minutes before repeating synaptic measurements. In other experiments TFS was applied by switching to voltage-clamp and stimulating 300 times at 5 Hz at  $V_{\text{holding}} = -40$  mV. Single responses and trains were then re-measured in current clamp during a 10 min period immediately following TFS, and again in a 10 min period 30 min after TFS.

## Analysis

Data was analysed using WinLTP and Clampfit (Molecular Devices) and statistical analysis using SPSS 21 (IBM). Results are expressed as mean  $\pm$  SEM unless indicated otherwise. Statistical analysis of current clamp data was performed using repeated measures ANOVA or paired t-test. Statistical analysis of plasticity experiments was assessed by averaging data points from the last 15 min of the experiment using one-way ANOVA with Bonferroni post-hoc comparisons to allow inclusion of a control data group to account for effects of rundown. EPSC<sub>NMDA</sub> decay time constants were fit by a double exponential function using Clampfit and a weighted time constant ( $\tau_w$ ) calculated as  $\tau_w = \tau_{\text{fast}} * (A_{\text{fast}} / (A_{\text{fast}} + A_{\text{slow}})) + \tau_{\text{slow}} * (A_{\text{slow}} / (A_{\text{slow}} + A_{\text{fast}}))$  where A = amplitude.  $\tau_w$  were compared using paired t-tests. Statistical significance was set at 0.05 for all comparisons. Statistical analysis of the effects of hippocampal lesions on electrophysiology were performed using Mann Whitney non-parametric test.

## Figure Legends

### **Figure S1. Recording of hippocampal-PFC synaptic transmission in the rat in vitro.**

Following labelling of the ventral hippocampal CA1 region with an anterograde tracer **(A)**, fibres were observed running in a ventral-dorsal course **(B, bottom right)** and innervated infralimbic and prelimbic cortex **(B, top right)**. Stimulation electrode is placed within hippocampal fibre bundle and recordings made from prelimbic layer 5. **(C)** To confirm the origin of the putative hippocampal fibre bundle, unilateral excitotoxic lesions were made in 6 animals. 21 days later volumetric lesion analysis showed  $61 \pm 8$  % of hippocampal volume was lost. Largest (grey) and smallest (black) lesion volumes are shown. **(D)** In PFC slices contralateral to the lesion, stimulation of hippocampal afferents evoked large EPSCs in all cells (mean amplitude  $299 \pm 45$  pA,  $n = 17$  cells from 6 animals) whereas in slices from the ipsilateral hemisphere responses were absent or profoundly reduced in amplitude ( $38 \pm 8.4$  pA,  $n = 19$  cells from 6 animals) when compared to the contralateral hemisphere (Mann-Whitney test, \*\*\* =  $p < 0.001$ ).

**Figure S2. D2-like dopamine receptor activation does not regulate basal NMDA transmission nor expression of NMDA-LTD and induction of NMDA-LTD does not require activation of NMDA receptors.** **(A)** Bath application of sulpiride (10  $\mu$ M) had no effect on basal EPSC<sub>NMDA</sub> transmission ( $93.8 \pm 4.5$  % of baseline,  $n = 5$ , paired t-test  $t_{(4)} = -1.127$ ,  $p = 0.32$ ). **(B)** The maintenance of LTD of EPSC<sub>NMDA</sub> ( $45.2 \pm 8.2$  % of baseline,  $n = 6$ ) is unaffected by sulpiride (10  $\mu$ M), when bath applied 30 minutes later ( $44.7 \pm 10.3$  % of baseline, paired t-test  $t_{(5)} = 0.192$ ,  $p = 0.86$ ). **(C)** Hyperpolarisation of cell to -100 mV during TFS (shaded region) prevented ionic conductance through NMDA receptors (dark grey trace, inset) but did not prevent induction of NMDAR-LTD (filled circles,  $57.4 \pm 5.5$  % of baseline,  $n = 5$ , one-way ANOVA, Bonferroni post hoc vs TFS,  $p = 1.0$ ). Hyperpolarisation to -100 mV in the

absence of TFS was not sufficient to induce NMDAR-LTD (open circles,  $87.1 \pm 8.8$  %,  $n = 4$ , post hoc vs TFS  $p = 0.002$ , post hoc vs control  $p = 1.0$ ). See Fig. 2E for summary. **(D)** Bath application of AP5 (50  $\mu$ M, shaded region) before delivery of TFS did not prevent induction of LTD (filled circles,  $56.4 \pm 4.8$  %,  $n = 8$ , Bonferroni post vs TBS alone  $p = 1.0$ , Control  $p < 0.001$ ). Furthermore TFS applied in the presence of AP5 was significantly different to a control group AP5 applied in the absence of TFS (open circles,  $74.6 \pm 2.7$  %,  $n = 8$ , Bonferroni post hoc,  $p = 0.041$ ). The AP5 control group was not significantly different to time matched controls ( $p = 0.11$ ). See Fig. 2E for summary. Insets show representative traces at baseline (black) and 46-60 mins (grey).

**Figure S3. (A)** D1R-like dopamine receptor agonist SKF81297 (0.5  $\mu$ M) had no effect on EPSC<sub>NMDA</sub> ( $98.9 \pm 8.6$  % of baseline,  $n = 5$ , one way-ANOVA, Bonferroni vs control  $p = 1.0$ , vs TFS  $p < 0.001$ ). Bath application of muscarinic acetylcholine receptor antagonists **(B)** atropine (1  $\mu$ M,  $52.7 \pm 4.6$  %,  $n = 5$ ; post hoc vs TFS alone  $p = 1.0$ ) or **(C)** scopolamine (10  $\mu$ M,  $55.2 \pm 9.4$  %  $n = 6$ ; post hoc vs TFS alone  $p = 1.0$ ) did not prevent induction of LTD by TFS. **(D)** Bath application of LY341495 at a concentration (100  $\mu$ M) that acts as a broad spectrum mGluR antagonist did not prevent activity-dependent NMDAR-LTD ( $70.1 \pm 4.6$  %,  $n = 5$ , post hoc vs TFS alone  $p = 1.0$ ). **(E)** LTD of EPSC<sub>NMDA</sub> by TFS ( $64.8 \pm 2.1$ %,  $n = 5$ , light grey trace) was reversed by bath application of forskolin (10  $\mu$ M, dark grey trace,  $90.8 \pm 10.8$  % of baseline, paired t-test  $t_{(4)} = -2.79$ ,  $p = 0.0495$ ). **(F)** NMDAR-LTD induced by TFS was not blocked by bath application of GSK3 $\beta$  antagonists TDZD-8 (10  $\mu$ M;  $58.5 \pm 7.1$  %,  $n = 6$ ; Bonferroni vs TFS:  $p = 1.0$ ) or SB216763 (10  $\mu$ M,  $60.7 \pm 5.6$  %,  $n = 9$ , post hoc vs TFS alone  $p = 1.0$ ). Insets show representative traces at baseline (black) and 46-60 mins (grey). **(A-D)** are summarised in Fig. 2F).

**Figure S4. Effects of NMDA receptor modulation on action potential firing. (A)** Left: plot of action potentials fired during synaptic bursts at different frequencies before and after bath application of AP5 (50  $\mu$ M), averaged across 3 cells. Right: example trace of a cell that fires in response to a 20 Hz synaptic burst before, but not after AP5. **(B)** Left: action potentials fired before and after TFS averaged across 4

cells. Right: Example traces of cell spiking in response to a 50 Hz synaptic burst with spiking attenuated following TFS. **(C)** Left: A single cell fired in response to 50 and 100 Hz synaptic bursts both before and after delivery of TFS in the presence of sulpiride. Right: example traces of 50 Hz bursts before and after TFS + sulpiride. Due to low experimental number statistical analyses were not performed on these data.









